

**REMARKS**

Entry of the foregoing, reexamination, and reconsideration of the instant application, pursuant to and consistent with 37 C.F.R. § 1.116, are respectfully requested in light of the remarks which follow.

**Status**

As is correctly reflected in the Office Action Summary, prior to the instant Response Claims 7, 15, 16, 18, 19, and 41-75 were pending. *Office Action mailed July 11, 2011, Office Action Summary, Item 4.* Claims 46-75 were withdrawn from consideration. *Id. at Item 4a.* Claims 7, 15, 16, 18, 19, and 41-45 were rejected. *Id. at Item 6.*

**Summary Of Amendments**

By the foregoing amendments, Applicants amended Claims 7, 15, 18, and 41 to recite an isolated *Escherichia coli*. Support for these amendments may be found throughout the Specification, and at least at original Claim 16. Accordingly, no new matter has been added.

Further by the foregoing amendments, Applicants amended Claim 7 to specify that the NADH-dependent D-lactate dehydrogenase activity is enhanced. Support for this amendment may be found throughout the Specification, and at least at Page 14, Lines 8-12. Accordingly, no new matter has been added.

Finally by the foregoing amendments, Applicants canceled Claims 16 and 42-45 without prejudice or disclaimer to Applicants filing one or more continuing applications directed to the previously-presented subject matter.

### **Rejections Under 35 U.S.C. § 101 – Non-Statutory Subject Matter**

The Office Action mailed on July 11, 2011, is unclear as to whether Claims 7, 15, 16, 18, 19, and 41-45 were rejected under 35 U.S.C. § 101 as purportedly containing non-statutory subject matter. *Office Action mailed July 11, 2011, Pages 2-3*. If these rejections exist, these rejections are respectfully traversed.

Not to acquiesce in the Examiner's rejections, but solely to facilitate prosecution, by the foregoing amendments Claim 7 was amended to specify that said NADH-dependent D-lactate dehydrogenase activity is enhanced.

Applicants believe these amendments have rendered moot the Examiner's possible rejections, and respectfully request withdrawal thereof.

### **Rejections Under 35 U.S.C. § 103 – Zhou, Yang, and Shaw**

Claims 7, 15, 16, 18, 42, and 44 remain rejected under 35 U.S.C. § 103(a) as purportedly unpatentable over Zhou *et al.*, *Production of Optically Pure D-Lactic Acid in Mineral Salts Medium by Metabolically Engineered Escherichia coli W3110*, 69(1) APPLIED AND ENVIRONMENTAL MICROBIOLOGY 399-407 (2003) ("Zhou") in view of Yang *et al.*, *Redistribution of Metabolic Fluxes in Escherichia coli with Fermentative Lactate Dehydrogenase Overexpression and Deletion*, 1 METABOLIC ENGINEERING 141-152 (1999) ("Yang") and Shaw *et al.*, *Vinylglycolate Resistance in Escherichia coli*, 121(3) J.

BACTERIOLOGY 1047-1975 (1975) ("Shaw"). *Office Action mailed July 11, 2011, Pages 3-11*. These rejections are respectfully traversed.

"A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains." 35 U.S.C. § 103(a).

Underlying factual determinations in an obviousness analysis include (1) the scope and content of the prior art, (2) the level of ordinary skill in the art, (3) the differences between the claimed invention and the prior art, and (4) objective indicia of nonobviousness. *Merck & Co. v. Teva Pharms. USA, Inc.*, 395 F.3d 1364, 1369 (Fed. Cir. 2005) (citing *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966)).

Applicants respectfully submit that a *prima facie* case of obviousness against Claims 7, 15, 18, and 44 due to Zhou, Yang, and Shaw has not been made.

At the outset, it is important to recognize that each of Claims 7, 15, 18, and 44 must be considered *as a whole*. See, e.g., *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1742 (2007) ("What matters is the objective reach of the claim."); *Datascope Corp. v. SMEC, Inc.*, 776 F.2d 320, 324 (Fed. Cir. 1985) (noting that the references must be considered "as a whole" and that the claimed subject matter must be considered "as a whole."). Considering each of Claims 7, 15, 18, and 44 as a whole is important because "a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." *KSR*, 127 S. Ct. at 1741. That is because "inventions in most, if not all,

instances rely upon building blocks long since uncovered, and claimed discoveries almost of necessity will be combinations of what, in some sense, is already known.” *Id.*

Amended independent Claim 7, as a whole, is directed to:

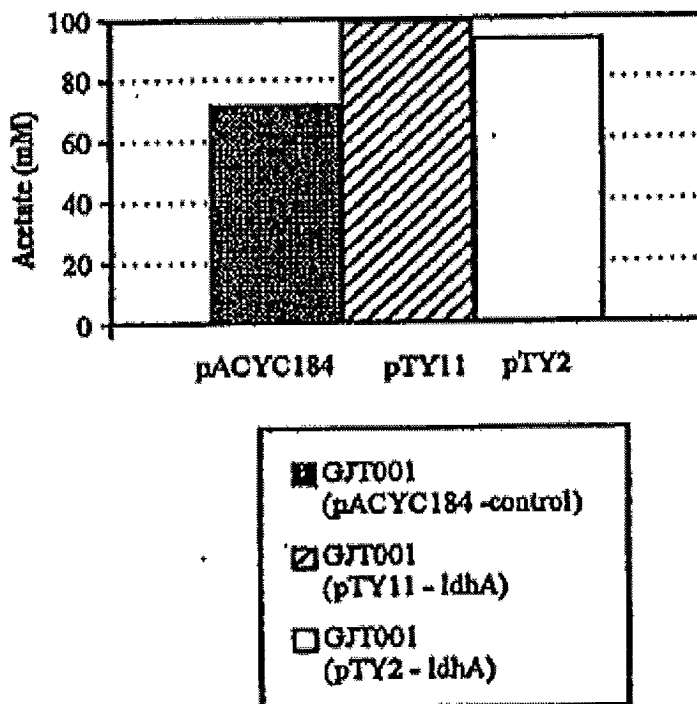
An isolated *Escherichia coli* comprising an NADH-dependent D-lactate dehydrogenase (ldhA) gene obtained from *Escherichia coli* wherein said *Escherichia coli*'s FAD-dependent D-lactate dehydrogenase (dld) inherent activity is inactivated or decreased, wherein said *Escherichia coli*'s pyruvate formate-lyase (pfl) inherent activity is inactivated or decreased, and wherein said NADH-dependent D-lactate dehydrogenase activity is enhanced.

In issuing the obviousness rejections, as opposed to anticipation rejections, the Examiner admits that neither Zhou, Yang, nor Shaw alone disclose each and every element of Claim 7. The Examiner also admits that Zhou “does not specifically teach a metabolic engineering [of] *E. coli* with reduced activity in FAD-dependent D-lactate dehydrogenase (*dld*).” *Office Action mailed July 11, 2011, Page 4*. Thus, it becomes “important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does.” *KSR*, 127 S. Ct. at 1741.

Applicants respectfully submit that a reason to vary from Zhou has not been provided nor has a sufficient reason been provided to combine *select* aspects of Zhou with *select* aspects of Yang and Shaw. The Examiner admits that “metabolic engineering is a complex science” yet claims that “it would have been *prima facie* obvious in an attempt to provide enhanced production of D-lactic acid, to metabolically [engineer] *E. coli* to study how the balance flux from pyruvic acid to lactate is modified.” *Office Action mailed July 11, 2011, Page 6*.

In addition to reiterating the arguments provided in Applicants' Response filed on May 31, 2011, Applicants respectfully submit that one of ordinary skill in the art would not arrive at Applicants' claimed invention based upon Zhou, Yang, and Shaw.

Figure 4 of Yang indicates that the yield of acetate from the mutants having enhanced *ldhA* activity (pTY11 and pTY2) increases, as compared with the control (pACYC184):



Yang, Page 148. Yang also states:

**The positive amplification factors (or deviation indices) for all three branches implies that an increase in the LDH**

**activity not only increases its own flux and the common flux, but also increases the flux of the competing branch. These results are quite different from a branch point with normal enzyme kinetics, where a flux amplification in one branch often leads to a decrease in the competing branch.**

*Yang at Pages 148, 150.* Thus, Yang teaches that the mutations of the enzyme in the anaerobic metabolic pathway of *E. coli* give results *unexpected* from normal enzyme kinetics. Yang specifically teaches that mutants having the enhanced IdhA activity increases the yield of acetic acid. This is also supported by Table 23 in Applicants' Specification. In Table 23, the amount of acetic acid from the mutant having enhanced IdhA activity, Strain E, increases to 7.0 g/L as compared with Strain C, which has normal IdhA activity and 4.4 g/L acetic acid:

	MG1655Δpfl Strain C	MG1655Δpfl/pGlyldhA Strain E	MG1655ΔpflΔdld/pGlyldhA Strain F
D-Lactic Acid	60 g/L	43 g/L	65 g/L
Pyruvic Acid	2.3 g/L	0.9 g/L	0.7 g/L
Formic Acid	ND	3.5 g/L	ND
<b>Acetic Acid</b>	<b>4.4 g/L</b>	<b>7.0 g/L</b>	<b>4.2 g/L</b>

*See Specification, Page 57.* Yet, surprisingly, Strain F, having enhanced IdhA with deleted dld activity, has a reduced amount of acetic acid – 4.2 g/L compared with 4.4 g/L for Strain C. Thus, Strain F contradicts Yang's teaching that a mutant with enhanced IdhA activity will have increased acetic acid yield. As such, even if it is determined that a *prima facie* case of obviousness exists due to Zhou, Yang, and Shaw, the unexpected results associated with Applicants' claimed invention rebuts such a showing. See, e.g., *In re Sullivan*, 498 F.3d 1345, 1351 (Fed. Circ. 2007).

Because Zhou, Yang, and Shaw, either alone or in hindsight-based combination, fail to disclose or suggest Applicants' claimed invention and especially in view of the unexpected results associated with Applicants' claimed invention, Applicants respectfully request withdrawal of the rejection of Claims 7, 15, 18, and 44 under 35 U.S.C. § 103(a).

**Rejections Under 35 U.S.C. § 103 – Zhou, Yang, Shaw, and Courtright**

Claim 41 remains rejected under 35 U.S.C. § 103 as purportedly being unpatentable over Zhou in view of Yang and Shaw, further in view of Courtright *et al.*, *Malate Dehydrogenase Mutants in Escherichia coli K-12*, 102(3) *J. Bacteriol.* 722-728 (1970) ("Courtright"). *Office Action mailed July 11, 2011, Pages 11-12.* These rejections are respectfully traversed.

The ways in which Zhou, Yang, and Shaw fail to render, *inter alia*, Claim 7 obvious are detailed above. Courtright fails to cure the deficiencies of Zhou, Yang, and Shaw. At best, Courtright pertains to mutants isolated from *Escherichia coli* K-12 who are "devoid of malate dehydrogenase activity." *Courtright*, Page 722. Again, references must be considered "as a whole" and the claimed subject matter must be considered "as a whole." *Datascope, Inc.*, 776 F.2d at 324. Factfinders must avoid the distortion caused by hindsight bias "and must be cautious of arguments reliant upon *ex post* reasoning." *KSR*, 127 S. Ct. at 1742 (citing *Graham* and "warning against a 'temptation to read into the prior art the teachings of the invention in issue' and instructing courts to 'guard against slipping into the use of hindsight.'"). Applicants submit that nothing, other than Applicants' claimed invention, discloses the subject matter of Claim 41. Courtright

itself is silent as to Claim 41's requirement that the microorganism's aspartate ammonia-lyase activity is inactivated or decreased.

Moreover, Claim 41 depends from independent Claim 7. "If an independent claim is nonobvious under 35 U.S.C. [§] 103, then any claim depending therefrom is nonobvious. *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988)." *Manual of Patent Examining Procedure* ("M.P.E.P."), § 2143.03. Because Claim 7 is nonobvious, Claim 41 is similarly nonobvious.

Accordingly, Applicants respectfully request withdrawal of the rejection of Claim 41 under 35 U.S.C. § 103(a) over Zhou, Yang, Shaw, and Courtright.

#### **Rejections Under 35 U.S.C. § 103 – Zhou, Yang, Shaw, and Maier**

Claims 18 and 19 remain rejected under 35 U.S.C. § 103 as purportedly being unpatentable over Zhou in view of Yang and Shaw, and further in view of U.S. Patent Publication No. 2004/0038352, published on February 26, 2004, to Maier ("Maier"). *Office Action mailed July 11, 2011, Pages 12-13*. These rejections are respectfully traversed.

The ways in which Zhou, Yang, and Shaw fail to render, *inter alia*, Claim 7 obvious are detailed above. Claim 15 depends from Claim 7 and specifies that said *ldhA* gene expresses the *ldhA* on the genome of the *Escherichia coli* by using a promoter of a gene which controls expression of a protein involved in a glycolytic pathway, a nucleic acid biosynthesis pathway, or an amino acid biosynthesis pathway. Maier fails to cure the deficiencies of Zhou, Yang, and Shaw. At best, Maier pertains to a short list of promoters for increasing expression of a desired gene. See, e.g., Maier at

[0030]-[0031]. Use of a promoter does not remove the complexity and uncertainty associated with Applicants' invention, as detailed above with regard to Yang.

Moreover, references must be considered "as a whole" and the claimed subject matter must be considered "as a whole." *Datascope, Inc.*, 776 F.2d at 324. Factfinders must avoid the distortion caused by hindsight bias "and must be cautious of arguments reliant upon *ex post* reasoning." *KSR*, 127 S. Ct. at 1742 (citing *Graham* and "warning against a 'temptation to read into the prior art the teachings of the invention in issue' and instructing courts to 'guard against slipping into the use of hindsight.'"). Applicants submit that nothing, other than Applicants' claimed invention, discloses the subject matter of Claims 18 and 19. Maier itself is silent as to the particular attributes of Claim 18 and Claim 19.

In addition, Claims 18 and 19 depend, indirectly, from independent Claim 7. "If an independent claim is nonobvious under 35 U.S.C. [§] 103, then any claim depending therefrom is nonobvious. *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988)." *Manual of Patent Examining Procedure* ("M.P.E.P."), § 2143.03. Because Claim 7 is nonobvious, Claims 18 and 19 are similarly nonobvious.

Accordingly, Applicants respectfully request withdrawal of the rejection of Claims 18 and 19 under 35 U.S.C. § 103(a) over Zhou, Yang, Shaw, and Maier.

#### **Rejections Under 35 U.S.C. § 112, Second Paragraph – Indefiniteness**

Claims 7, 15, 16, 18, 19, and 41-45 were rejected under 35 U.S.C. § 112, Second Paragraph, as purportedly indefinite. *Office Action mailed July 11, 2011, Pages 13-14*. These rejections are respectfully traversed.

Not to acquiesce in the Examiner's rejections, but solely to facilitate prosecution, by the foregoing amendments Applicants amended independent Claim 7, from which Claims 15, 18, 19, and 41 depend (either directly or indirectly), to read "wherein said NADH-dependent D-lactate dehydrogenase activity is enhanced."

Applicants believe this amendment has rendered moot the Examiner's rejections, and respectfully request withdrawal thereof.

**Request for Rejoinder**

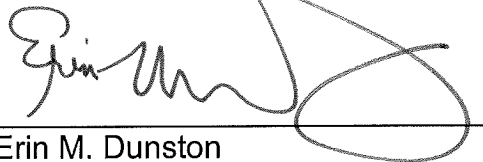
Withdrawn Claims 46-75 are method claims that depend, directly or indirectly, from allowable independent Claim 7. Pursuant to at least M.P.E.P. § 821.04, Applicants respectfully request that Claims 46-75 be rejoined with allowable Claims 7, 15, 18, 19, and 41.

**CONCLUSION**

In the event that there are any questions relating to this Amendment and Reply To Final Office Action Pursuant To 37 C.F.R. § 1.116, or to the application in general, it would be appreciated if the Examiner would contact the undersigned attorney by telephone at (703) 836-6620 so that prosecution of the application may be expedited.

The Patent Office is hereby authorized to charge any necessary fees, or credit any overpayment, to Deposit Account No. 02-4800.

Respectfully submitted,  
BUCHANAN INGERSOLL & ROONEY PC

A handwritten signature in black ink, appearing to read "Erin M. Dunston", written over a horizontal line.

Date: October 11, 2011

By:

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